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E6	1	AU=TOMASSINI, JOANNE ELIZABETH
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S1 1 AU="TOMASSINI, JOANNE ELIZABETH"  
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933408 ORDER NO: AAD86-24033  
ISOLATION, CHARACTERIZATION AND CLONING OF THE CELLULAR RECEPTOR FOR THE  
MAJOR GROUP OF HUMAN RHINOVIRUSES (COMMON COLD)  
Author: **TOMASSINI, JOANNE ELIZABETH**  
Degree: PH.D.  
Year: 1986  
Corporate Source/Institution: UNIVERSITY OF PENNSYLVANIA (0175)  
Source: VOLUME 47/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.  
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933408 ORDER NO: AAD86-24033  
ISOLATION, CHARACTERIZATION AND CLONING OF THE CELLULAR RECEPTOR FOR THE  
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Author: **TOMASSINI, JOANNE ELIZABETH**  
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Source: VOLUME 47/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.  
PAGE 2774. 151 PAGES

Viruses attach to specific receptors on host cells as the first step in viral pathogenesis. Characterization of the host-virus interaction requires isolation of the host cell receptor and virus attachment protein. Human rhinoviruses (HRVs) can be classified into major and minor groups on the basis of receptor specificity. Recently, a mouse monoclonal antibody was isolated which selectively blocked the attachment of the major group of HRVs to cells. Using this antibody, the cellular receptor protein for the major group of HRVs was isolated from host cells. First, a radioimmunoassay was developed using the receptor antibody to specifically detect detergent-solubilized receptor during isolation. A receptor protein, with a molecular weight of 90,000 on SDS polyacrylamide gels, was then purified from detergent-treated HeLa cell membranes on an immunoaffinity column containing the anti-receptor antibody. Polyclonal rabbit antiserum, resulting from immunization with the isolated receptor protein, specifically blocked the attachment of the major group of HRVs and exhibited the same specificity as the anti-receptor monoclonal antibody. Neuraminidase digestion of the isolated receptor protein demonstrated that the receptor protein is a glycoprotein containing sialic acid at the non-reducing ends of the carbohydrate. Digestion with N-glycanase resulted in a molecular weight shift to 60 kDa on SDS polyacrylamide gels, presumably the deglycosylated form of the receptor protein.

The receptor polyclonal antiserum was used to clone the HRV receptor gene from a human skin epidermal cDNA library. Two clones were isolated which were antibody positive after three rounds of plaque purification. The fusion protein expressed by one of these clones was characterized by several techniques including epitope selection, competition RIA and the preparation of antiserum to the protein, and was shown to contain an epitope which corresponds to the receptor protein. In addition, Northern gel analysis of various human cell lines probed with the DNA from this clone resulted in detection of a 2.7Kb mRNA species; however, mRNA was not detected in rodent cells which lack the HRV receptor. Additional cDNA clones were identified in the same cDNA library by hybridization with this DNA probe. Two of these clones have been shown to overlap and may represent the entire length of the HRV receptor gene mRNA.

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05479598 BIOSIS NO.: 000033080451  
 CHARACTERIZATION OF THE CELLULAR RECEPTOR SPECIFIC FOR ATTACHMENT OF MOST  
 HUMAN RHINOVIRUS SEROTYPES  
 AUTHOR: COLONNO R J; **TOMASSINI J E**; CALLAHAN P L; LONG W J  
 AUTHOR ADDRESS: VIRUS CELL BIOL. RES., MERCK SHARP AND DOHME RES. LAB.,

WEST POINT, PA. 19486.  
JOURNAL: CROWELL, R. L. AND K. LONBERG-HOLM (ED.). VIRUS ATTACHMENT AND ENTRY INTO CELLS; ASM (AMERICAN SOCIETY FOR MICROBIOLOGY) CONFERENCE, PHILADELPHIA, PENNSYLVANIA, USA, APRIL 10-13, 1985. VIII+216P. ASM: WASHINGTON, D.C., USA. ILLUS. PAPER. ISBN 0-914826-90-5. 0 (0). 1986 (RECD. 1987). 109-115.  
CODEN: 25817  
RECORD TYPE: Citation  
LANGUAGE: ENGLISH

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05184455 BIOSIS NO.: 000082025076  
ISOLATION OF A RECEPTOR PROTEIN INVOLVED IN ATTACHMENT OF HUMAN RHINOVIRUSES  
AUTHOR: **TOMASSINI J E**; COLONNO R J  
AUTHOR ADDRESS: DEP. OF VIRUS AND CELL BIOL., MERCK SHARP AND DOHME RES. LAB., WEST POINT, PA. 19486.  
JOURNAL: J VIROL 58 (2). 1986. 290-295.  
FULL JOURNAL NAME: Journal of Virology  
CODEN: JOVIA  
RECORD TYPE: Abstract  
LANGUAGE: ENGLISH

ABSTRACT: Human rhinoviruses can be classified into major and minor groups on the basis of receptor specificity. Recently, a mouse monoclonal antibody was isolated which selectively blocked the attachment of the major group of human rhinoviruses to cells. Using this monoclonal antibody, the cellular receptor for the major group of human rhinovirus was isolated. A radioimmunoassay was developed by using the receptor antibody to specifically detect rhinovirus receptor during isolation. Solubilized receptor from detergent-treated HeLa cell membrane extracts eluted from gel filtration columns with an apparent molecular-weight of 440,000. A cellular receptor protein, which had a molecular weight of 90,000 when analyzed on sodium dodecyl sulfate-polyacrylamide gels, was purified from solubilized extracts on an immunoaffinity column containing receptor antibody. Polyclonal rabbit antiserum, resulting from immunization with the isolation receptor protein, specifically blocked the attachment of the major group of human rhinoviruses and indicated that the 90-kilodalton protein plays a functional role in attachment. Prolonged exposure of HeLa cell monolayers with the receptor antibody showed no inhibition of cell growth and division.

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05007979 BIOSIS NO.: 000031083111  
ISOLATION AND CHARACTERIZATION OF A CELLULAR RECEPTOR INVOLVED IN ATTACHMENT OF HUMAN RHINOVIRUSES TO CELLS  
AUTHOR: **TOMASSINI J E**; COLONNO R J  
AUTHOR ADDRESS: MERCK SHARP AND DOHME RESEARCH LABORATORIES, WEST POINT, PA 19486.  
JOURNAL: SYMPOSIUM ON POSITIVE STRAND RNA VIRUSES HELD AT THE 15TH ANNUAL MEETING OF THE UCLA (UNIVERSITY OF CALIFORNIA-LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, APR. 20-26, 1986. J CELL BIOCHEM SUPPL 0 (10 PART D). 1986. 300.  
CODEN: JCBSD  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
LANGUAGE: ENGLISH

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05007891 BIOSIS NO.: 000031083023  
HUMAN RHINOVIRUS ATTACHMENT REQUIRES A SPECIFIC CELLULAR RECEPTOR PROTEIN  
AUTHOR: COLONNO R J; **TOMASSINI J E**; CALLAHAN P L  
AUTHOR ADDRESS: MERCK SHARP AND DOHME RESEARCH LABORATORIES, WEST POINT, PA  
19486.  
JOURNAL: SYMPOSIUM ON POSITIVE STRAND RNA VIRUSES HELD AT THE 15TH ANNUAL  
MEETING OF THE UCLA (UNIVERSITY OF CALIFORNIA-LOS ANGELES) SYMPOSIA ON  
MOLECULAR AND CELLULAR BIOLOGY, APR. 20-26, 1986. J CELL BIOCHEM SUPPL 0  
(10 PART D). 1986. 266.  
CODEN: JCBSD  
DOCUMENT TYPE: Meeting  
RECORD TYPE: Citation  
LANGUAGE: ENGLISH

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DIALOG(R) File 155:MEDLINE(R)  
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06034650 86200369

Isolation of a receptor protein involved in attachment of human rhinoviruses.

Tomassini JE; **Colonno RJ**

Journal of virology (UNITED STATES) May 1986, 58 (2) p290-5,  
ISSN 0022-538X Journal Code: KCV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Human rhinoviruses can be classified into major and minor groups on the basis of receptor specificity. Recently, a mouse monoclonal antibody was isolated which selectively blocked the attachment of the major group of human rhinoviruses to cells. Using this monoclonal antibody, the cellular receptor for the major group of human rhinoviruses was isolated. A radioimmunoassay was developed by using the receptor antibody to specifically detect rhinovirus receptor during isolation. Solubilized receptor from detergent-treated HeLa cell membrane extracts eluted from gel filtration columns with an apparent molecular weight of 440,000. A cellular receptor protein, which had a molecular weight of 90,000 when analyzed on sodium dodecyl sulfate-polyacrylamide gels, was purified from solubilized extracts on an immunoaffinity column containing receptor antibody. Polyclonal rabbit antiserum, resulting from immunization with the isolated receptor protein, specifically blocked the attachment of the major group of human rhinoviruses and indicated that the 90-kilodalton protein plays a functional role in attachment. Prolonged exposure of HeLa cell monolayers with the receptor antibody showed no inhibition of cell growth and division.

5/7/2 (Item 2 from file: 155)  
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06029448 86115390

Monoclonal antibody that inhibits infection of HeLa and rhabdomyosarcoma cells by selected enteroviruses through receptor blockade.

Crowell RL; Field AK; Schleif WA; Long WL; **Colonno RJ**; Mapoles JE;  
Emini EA

Journal of virology (UNITED STATES) Feb 1986, 57 (2) p438-45,  
ISSN 0022-538X Journal Code: KCV

Contract/Grant No.: AI-03771, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

BALB/c mice were immunized with HeLa cells, and their spleen cells were fused with myeloma cells to produce hybridomas. Initial screening of culture fluids from 800 fusion products in a cell protection assay against coxsackievirus B3 (CB3) and the CB3-RD virus variant yielded five presumptive monoclonal antibodies with three specificities: protection against CB3 on HeLa, protection against CB3-RD on rhabdomyosarcoma (RD) cells, and protection against both viruses on the respective cells. Only one of the monoclonal antibodies (with dual specificity) survived two subclonings and was studied in detail. The antibody was determined to have an immunoglobulin G2a isotype and protected cells by blockade of cellular receptors, since attachment of [35S]methionine-labeled CB3 was inhibited by greater than 90%. The monoclonal antibody protected HeLa cells against infection by CB1, CB3, CB5, echovirus 6, and coxsackievirus A21 and RD

cells against CB1-RD, CB3-RD, and CB5-RD virus variants. The monoclonal antibody did not protect either cell type against 16 other immunotypes of picornaviruses. The monoclonal antibody produced only positive fluorescence on those cells which were protected against infection, and 125I-labeled antibody confirmed the specific binding to HeLa and RD cells. The results suggest that this monoclonal antibody possesses some of the receptor specificity of the group B coxsackieviruses.

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06027834 86089329

Isolation of a monoclonal antibody that blocks attachment of the major group of human rhinoviruses.

**Colonna RJ**; Callahan PL; Long WJ  
Journal of virology (UNITED STATES) Jan 1986, 57 (1) p7-12,  
ISSN 0022-538X Journal Code: KCV  
Languages: ENGLISH

Document type: JOURNAL ARTICLE

Reciprocal competition binding assays have previously demonstrated that 20 of 24 human rhinovirus serotypes tested compete for a single cellular receptor. These studies suggested that the vast majority of rhinovirus serotypes utilize a single cellular receptor. With HeLa cells as an immunogen, a mouse monoclonal antibody was isolated which had the precise specificity predicted by the competition binding study. The receptor antibody was shown to protect HeLa cells from infection by 78 of 88 human rhinovirus serotypes assayed. In addition, the receptor antibody protects HeLa cells from infection by three coxsackievirus A serotypes. The receptor antibody was unable to protect cells from infection by a wide range of other RNA and DNA viruses. Using the receptor antibody and human rhinovirus type 15, we determined that the cellular receptor utilized by the vast number of human rhinovirus serotypes is present only on cells of human origin, with the exception of chimpanzee-derived cells. The receptor antibody has a strong affinity for the cellular receptor as evidenced by its rapid binding kinetics and ability to displace previously bound human rhinovirus virions from receptors. No viral variants were identified which could bypass the receptor blockage.

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05802600 86089298

Use of monoclonal antibodies to identify four neutralization immunogens on a common cold picornavirus, human rhinovirus 14.

Sherry B; Mosser AG; **Colonna RJ**; Rueckert RR  
Journal of virology (UNITED STATES) Jan 1986, 57 (1) p246-57,  
ISSN 0022-538X Journal Code: KCV  
Contract/Grant No.: 5-T32-GM07215, GM, NIGMS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A collection of 35 mouse monoclonal antibodies, raised against human rhinovirus 14 (HRV-14), was used to isolate 62 neutralization-resistant mutants. When cross-tested against the antibodies in a neutralization assay, the mutants fell into four antigenic groups, here called neutralization immunogens: NIm-IA, -IB, -II, and -III. Sequencing the mutant RNA in segments corresponding to serotype-variable regions revealed that the amino acid substitutions segregated into clusters, which correlated exactly with the immunogenic groups (NIm-IA mutants at VP1 amino acid residue 91 or 95; NIm-II mutants at VP2 residue 158, 159, 161, or 162; NIm-III mutants at VP3 residue 72, 75, or 78; and NIm-IB mutants at two sites, either VP1 residue 83 or 85, or residue 138 or 139). Examination of the three-dimensional structure of the virus (M. G. Rossmann, E. Arnold, J.



W. Erickson, E. A. Frankenberger, J. P. Griffith, H.-J. Hecht, J. E. Johnson, G. Kamer, M. Luo, A. G. Mosser, R. R. Rueckert, B. Sherry, and G. Vriend, *Nature* [London], 317:145-153, 1985) revealed that each of the substitution clusters formed a protrusion from the virus surface, and the side chains of the substituted amino acids pointed outward. Moreover, four of the amino acid substitutions, which initially appeared to be anomalous because they were encoded well outside the cluster groups, could be traced to surface positions immediately adjacent to the appropriate viral protrusions. We conclude that three of the four antigens, NIm-IB, -II, and -III, are discontinuous. Thus, the amino acid substitutions in all 62 mutants fell within the proposed immunogenic sites; there was no evidence for alteration of any antigenic site by a distal mutation.

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105188553 CA: 105(21)188553w CONFERENCE PROCEEDING  
Characterization of the cellular receptor specific for attachment of most  
human rhinovirus serotypes  
AUTHOR(S): Colonno, Richard J.; Tomassini, Joanne E.; Callahan, Pia L.;  
Long, William J.  
LOCATION: Virus Cell Biol. Res., Merck Sharp and Dohme Res. Lab., West  
Point, PA, 19486, USA  
JOURNAL: Virus Attachment Entry Cells, Proc. ASM Conf. EDITOR: Crowell,  
Richard L. (Ed), Lonberg-Holm, Karl (Ed), DATE: 1986 PAGES: 109-15  
CODEN: 55GCAY LANGUAGE: English MEETING DATE: 850000 PUBLISHER: Am.  
Soc. Microbiol., Washington, D. C  
SECTION:  
CA114000 Mammalian Pathological Biochemistry  
CA110XXX Microbial Biochemistry  
IDENTIFIERS: review human rhino virus receptor  
DESCRIPTORS:  
Receptors...  
for rhino virus, on human cells  
Virus, animal, human rhino-...  
receptor for, on human cells

5/7/8 (Item 3 from file: 399)  
DIALOG(R)File 399:CA SEARCH(R)  
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105019852 CA: 105(3)19852n PATENT  
Human rhinovirus RNA and proteins  
INVENTOR(AUTHOR): Colonno, Richard J.; Mitzutani, Satoshi  
LOCATION: USA  
ASSIGNEE: Merck and Co., Inc.  
PATENT: European Pat. Appl. ; EP 169146 A2 DATE: 860122  
APPLICATION: EP 85401465 (850717) \*US 632785 (840720) \*US 721735 (850410)  
PAGES: 80 pp. CODEN: EPXXDW LANGUAGE: English CLASS: C12N-015/00A;  
C12N-005/00B; C12P-021/00B; C12P-021/00J; C12R-001/91J  
DESIGNATED COUNTRIES: AT; BE; CH; DE; FR; GB; IT; LI; LU; NL; SE  
SECTION:  
CA103004 Biochemical Genetics  
CA115XXX Immunochemistry  
CA163XXX Pharmaceuticals  
IDENTIFIERS: human rhinovirus genome cloning sequence, antibody human  
rhinovirus cDNA protein, cell receptor rhinovirus antibody  
DESCRIPTORS:  
Vaccines...  
against human rhinovirus, prepn. of, human rhinovirus-14 genome cloning  
in relation to  
Virus, animal, human rhinovirus 14...  
cloning and sequence of, antibody prepn. in relation to  
Escherichia coli...  
cloning in, of human rhinovirus-14 cDNA, antibody and vaccine prepn. in  
relation to  
Plasmid and Episome, pBR322...  
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relation to  
Gene and Genetic element, microbial...  
for human rhinovirus 14 proteins, cloning and nucleotide and encoded

peptide sequences of

Antibodies...

for human rhinovirus-14 or human rhinovirus-14 cell receptor antibody formation, viral genome cloning in relation to

Ribonucleic acid sequences,viral...

of human rhinovirus 14, complete

Molecular cloning...

of human rhinovirus-14 cDNA, in Escherichia coli, antibody and vaccine prepn. in relation to

Proteins,poly-...

of human rhinovirus-14, gene for, cloning and nucleotide and encoded peptide sequences of

Ribonucleic acids,viral...

of human rhinovirus-14, structure of

Protein sequences...

of protein VPg, of human rhinovirus 14, complete

Protein sequences...

of protein VP1, of human rhinovirus 14, complete

Protein sequences...

of protein VP2, of human rhinovirus 14, complete

Protein sequences...

of protein VP3, of human rhinovirus 14, complete

Protein sequences...

of protein VP4, of human rhinovirus 14, complete

Protein sequences...

of protein X, of human rhinovirus 14, complete

Protein sequences...

of protein 1B, of human rhinovirus 14, complete

Protein sequences...

of protein 3B, of human rhinovirus 14, complete

Protein sequences...

of protein 5B, of human rhinovirus 14, complete

Protein sequences...

of proteinase, of human rhinovirus 14, complete

Protein sequences...

of replicase, of human rhinovirus 14, complete

Antibodies,monoclonal...

to human rhinovirus 14 or human rhinovirus cell receptors, prepn. of, human rhinovirus 14 genome cloning in relation to

CAS REGISTRY NUMBERS:

94699-80-6 94699-88-4 94705-22-3 94716-26-4 94717-15-4 94717-16-5

94717-17-6 94717-18-7 96162-31-1 96162-44-6 96230-61-4 amino acid sequence of

94717-24-5 nucleotide sequence of

9031-50-9 94716-26-4 of human rhinovirus-14, cloning and nucleotide and amino acid sequence of cDNA for